IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Clarence N. Ahlem, et al.
Application No.	: 10/087,929
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Title	: Blood Cell Deficiency Treatment Method
Examiner	: James M. Spear
TC/A.U.	: 1618
Docket No.	: 202.8
Customer No.	: 26551
Confirmation No.	: 7989

DECLARATION

Mail Stop Amendment
Assistant Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Christopher L. Reading, declare as follows:

1. I am a co-inventor of the above-referenced patent application. I have been engaged in the evaluation and development of therapeutic agents and treatment methods for over 20 years, which includes 7 years of experience with preclinical and clinical development of steroid compounds. My curriculum vita is attached hereto. The following protocols were conducted under my supervision or with my knowledge.

2. Varying dosages and routes of administration for several compounds were tested for their capacity to treat radiation-induced cytopenia in rhesus macaque monkeys. The compounds were 3β,17β-dihydroxyandrost-5-ene (subcutaneous and intramuscular administration), 3β,7β,-trihydroxyandrost-5-ene (subcutaneous), 3β,17β-dihydroxy-17α-ethynylandrost-5-ene (oral), 3β-

hydroxy-17β-aminoandrost-5-ene (subcutaneous) and 3β-hydroxy-17β-aminoandrost-5-ene mesylate salt (oral and subcutaneous). The compounds were examined for their capacity to limit the duration of severe neutropenia (grade 4 neutropenia defined as an absolute neutrophil count of less than 500 per μL of blood) and thrombocytopenia after the animals were exposed to a non-lethal myelosuppressive dose of whole body γ-radiation. The radiation dose, 4 Gy, was administered on day 1 at a dose rate of about 0.6 Gy/minute as a single acute midline/bilateral whole body irradiation. This radiation dose usually causes a moderate to severe neutropenia, thrombocytopenia and/or anemia of varying duration. The severity and duration of the cytopenia depends in part on the individual animal's initial blood status. Other factors, such as an occult infection probably also affect an individual's initial blood status. Body weights were determined at least once prior to treatment, on the first day of treatment, and weekly thereafter. Compound doses were based on the most recently recorded body weight. Hematology samples from unfasted animals were drawn on multiple occasions during a 39-day period. Blood was drawn on days -3, 1, 3, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 30, 33 and 36. Blood was drawn before dosing on days when both dosing and blood draws were scheduled. Animal group designations and compound dose levels are shown in the table below. For the table below, compound #1 is 3β,17β-dihydroxyandrost-5-ene, compound #2 is 3β,7β,17β-trihydroxyandrost-5-ene, compound #3 is 3β,17β-dihydroxy-17α-ethynylandrost-5-ene, compound #4 is 3β-hydroxy-17β-aminoandrost-5-ene (free base) and compound #5 is 3β-hydroxy-17β-aminoandrost-5-ene mesylate salt. Vehicle (formulation) #1 in the table below was 3β-hydroxy-17β-aminoandrost-5-ene mesylate salt 440 mg/g, microcrystalline cellulose 490 mg/g, crossslinked povidone 50 mg/g and stearic acid 20 mg/g, vehicle #2 was 20 mg/mL of compound in 30% w/v cyclodextrin-sulfobutylether qs water, vehicle #3 was 0.1% w/v sodium carboxymethyl-cellulose + 0.9% w/v NaCl 2% w/v tween 80 and 0.05% w/v phenol, vehicle #4 was mesylate salt 2.66% w/v, 30% propylene glycol w/v, 2% benzyl alcohol w/v, pH 5, qs water and vehicle #5 was 20 mg/mL of compound in 400 mg/mL

hydroxypropyl β-cyclodextrin and 5 mg/mL sodium carboxymethylcellulose qs water. The formulations for groups 3, 7-9 and 12 were suspensions of the compound in vehicle and the formulation for groups 4, 10 and 11 were solutions.

Group No.	Animals M	Animals F	Compound #/ Vehicle #	Route	Dose (mg/kg)	Concentration (mg/mL)	Volume (mL/kg)	Administration Study day[a]
1	1	1	none (vehicle #1 control) / #1	PO	0[b]	0	0.25	1 through 20
2	1	1	none (vehicle #2 control) / #2	PO	0	0	0.33	1 through 20
3	2	1	#3 / #2	PO	3.3	10	0.33	1 through 20
4	2	1	#5 / #1	PO	5[b,c]	20	0.25	1 through 20
5	1	1	none (vehicle #3 control) / #3	SQ	0	0	0.25	1 through 10
6	1	1	none (vehicle #4 control) / #4	SQ	0[b]	0	0.120	1 through 20
7	1	1	#1 / #3	IM	15	60	0.25	1 through 5
8	2	1	#1 / #5	SQ	20[d]	20	1.00	1 through 20
9	2	1	#4 / #3	SQ	2.5[e]	20	0.125	1, 8, 15, 22
10	2	1	#5 / #4	SQ	2.5[c]	21	0.120	1, 8, 15, 22
11	2	1	#5 / #4	SQ	2.5[b,c]	21	0.120	1 through 20
12	2	1	#2 / #3	SQ	15	60	0.25	1 through 10

a Groups 1 through 12: The first dose was administered at 3 to 4 hours post-irradiation.

b Groups 1, 4, 6 and 11: Animals were dosed twice daily, 10 to 14 hours apart - the dose (mg/kg) was total daily dose.

c Total daily dose (mg/kg) based on the mesylate salt (free base equivalent was 4 mg/kg for Group 4 ; 2 mg/kg for Group 10, and 4 mg/kg for Group 11).

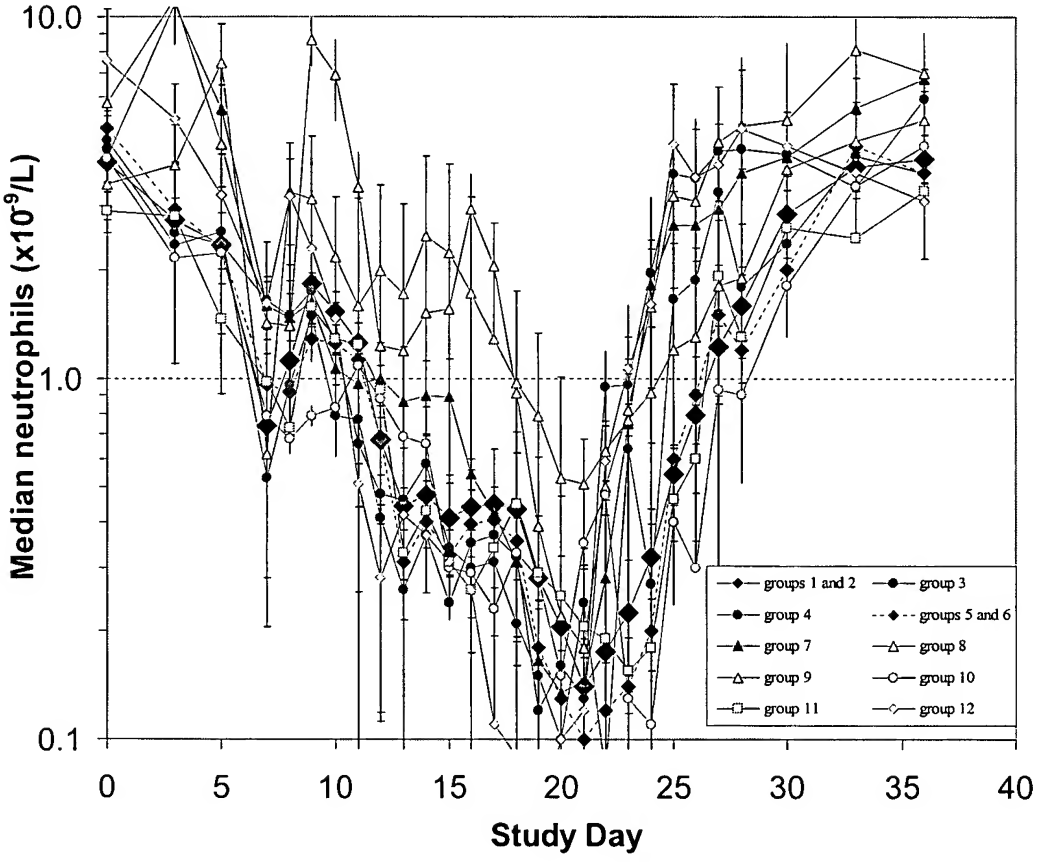
d Group 8: Animals were dosed 3 times daily at 6.5 to 9.5 hours apart - the dose (mg/kg) was the total daily dose.

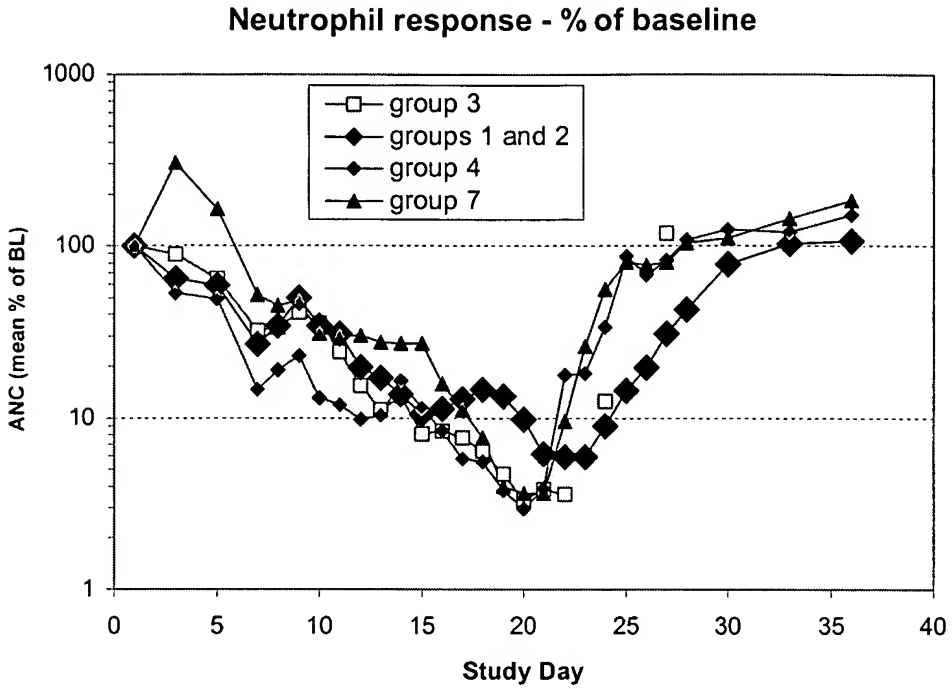
e Group 9: The dose was divided equally between 2 sites.

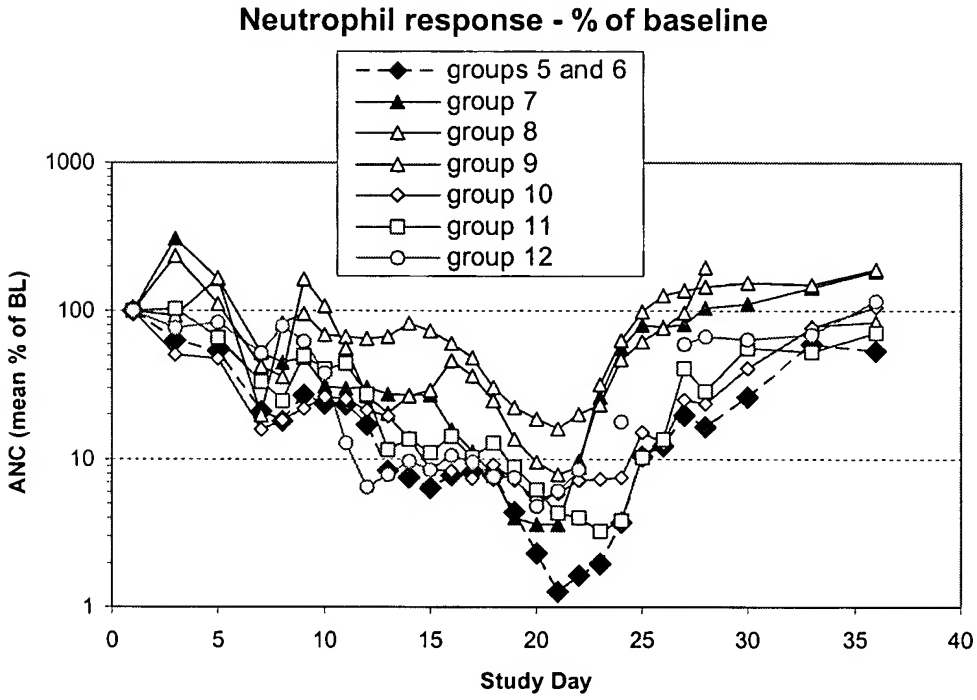
M = Male; F = Female; PO = *per os*; nasogastric intubation; SQ = subcutaneous injection; IM = intramuscular injection

3. The effect of the compound injections described in paragraph 2 for groups 5-12 ranged from significant injection site irritation (groups 4 and 10) and skin swelling (groups 7 and 8) to no apparent effect (e.g., groups 10 and 11). Results for the effect of the compound doses and dosing regimens described in paragraph 2 on the duration of grade 4 neutropenia (less than 0.5×10^3 neutrophils/μL) from the radiation exposure are summarized in the table and graphs shown below.

Group No.	Total days of severe Neutropenia (mean)	Mean day 1 neutrophil count (x $10^3/\mu L$)	Mean day 21 neutrophil count (x $10^3/\mu L$)	Administration Study day
1	10.5	4.06	0.14	1 through 20
2	7.5	5.16	0.40	1 through 20
3	10.0	5.17	0.18	1 through 20
4	11.7	6.61	0.19	1 through 20
5	7.0	6.25	0.10	1 through 10
6	10.0	6.58	0.10	1 through 20
7	6.5	4.69	0.15	1 through 5
8	2.7	3.75	0.58	1 through 20
9	3.0	7.93	0.38	1, 8, 15, 22
10	12.0	5.29	0.27	1, 8, 15, 22
11	10.0	2.88	0.21	1 through 20
12	10.7	10.97	0.16	1 through 10

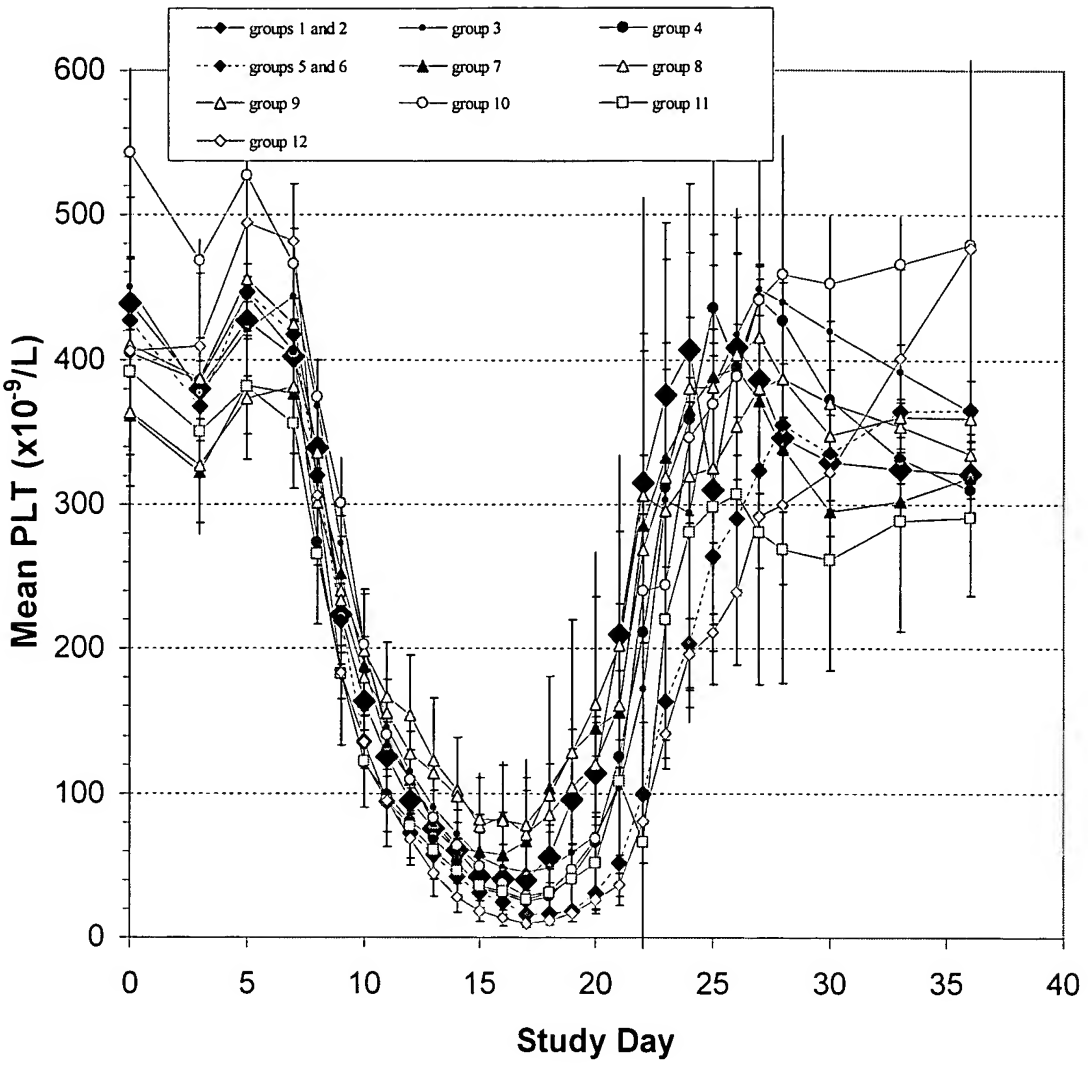



Neutrophil response - % of baseline
group 3
groups 1 and 2
group 4
group 7
ANC (mean % of BL)
1000
100
10
1
0
5
10
15
20
25
30
35
40
Study Day

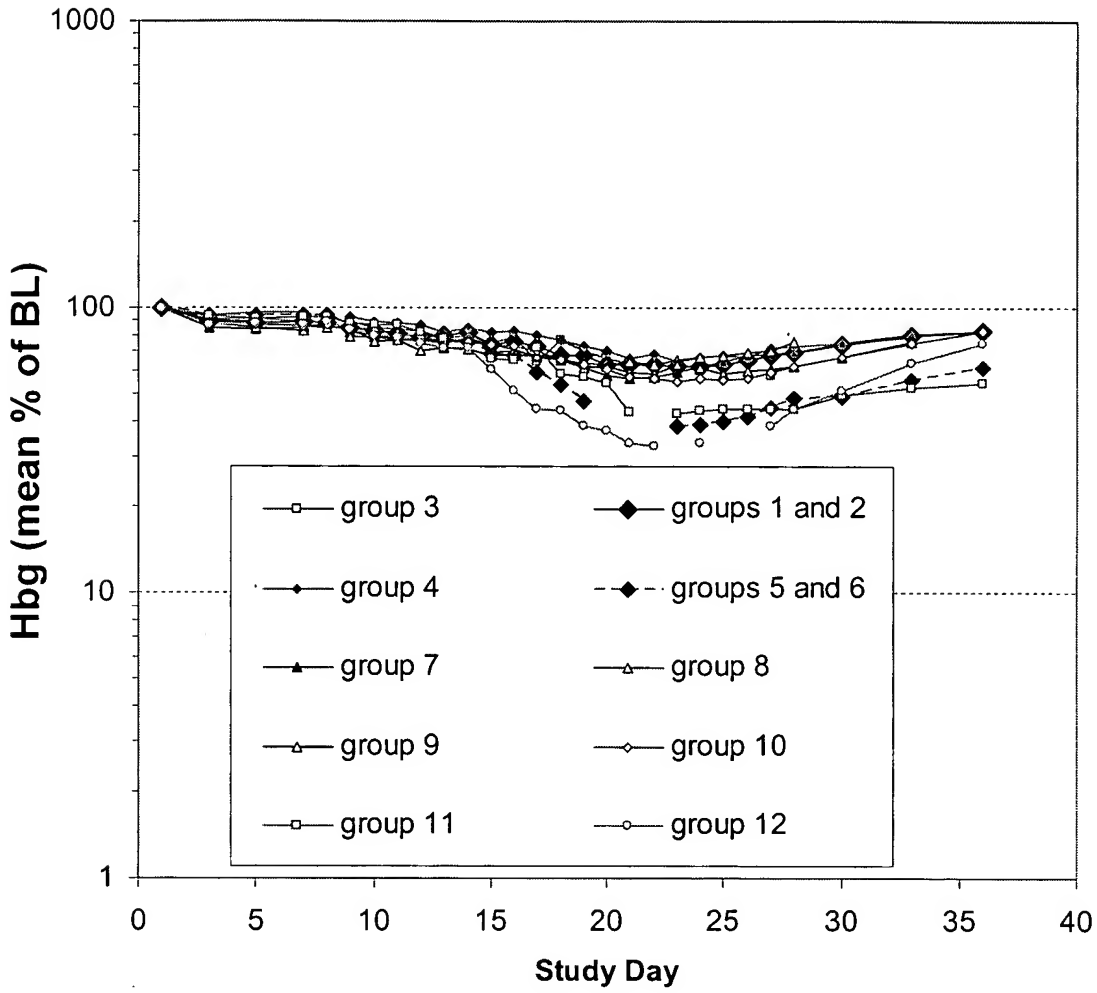

Neutrophil response - % of baseline
groups 5 and 6
group 7
group 8
group 9
group 10
group 11
group 12
ANC (mean % of BL)
1000
100
10
1
0
5
10
15
20
25
30
35
40
Study Day

4. Platelet counts (PLT) were measured in the irradiated animals described in paragraph 2 and the number of days of grade 4 thrombocytopenia (severe thrombocytopenia defined as less than 20 x 10^3 platelets/μL blood) were measured. At the 4 Gy radiation exposure that was used, the duration and/or severity of thrombocytopenia in the animals tended to be less compared to neutropenia in the animals. Results for the effect of the compound doses and dosing regimens described in paragraph 1 on thrombocytopenia (grade 4 thrombocytopenia defined as less than 20 x 10^3 platelets/μL) from the radiation exposure are summarized in the table and graph shown below.

Group No.	Thrombocytopenia Total days (mean)	Mean day 1 platelet count (x 10^3/μL)	Mean day 20 platelet count (x 10^3/μL)	Administration Study day
1	0.0	423	158	1 through 20
2	1.0	399	70	1 through 20
3	1.7	431	71	1 through 20
4	0.3	385	66	1 through 20
5	3.5	370	41	1 through 10
6	2.0	420	26	1 through 20
7	0.0	359	145	1 through 5
8	1.3	357	120	1 through 20
9	0.3	414	162	1, 8, 15, 22
10	0.7	522	69	1, 8, 15, 22
11	2.0	377	52	1 through 20
12	4.7	442	26	1 through 10



5. The occurrence and severity of anemia in the animals described in paragraph 2 was determined by measuring hemoglobin (Hbg) in the irradiated animals described in paragraphs 2 and 3. At the 4 Gy radiation exposure that was used, the duration and/or severity of anemia in the animals tended to be less pronounced compared to neutropenia in the animals. Results for the effect of the compound doses and dosing regimens described in paragraph 1 on the decrease in Hbg from the radiation exposure are summarized in the table and graph shown below.



6. Several compounds were tested for their capacity to treat chemotherapy-induced cytopenia in cynomolgus monkeys. The blood cell deficiencies were induced by treatment of the animals with a single 40 mg/kg dose of carboplatin. This dosage was sufficient to induce a pronounced cytopenia in most of the animals. The carboplatin was formulated at 10 mg/mL in aqueous 0.9% NaCl, USP for injection. Carboplatin was infused into the animals in a superficial vein over a period of about 30 minutes at 32-40 hours before the

first dose of compound was administered. For animals that received a compound, the compound dose was divided into two syringes containing approximately equal fractions, which were administered in two separate sites. The dosing time was the time of the completion of the second fraction administration. Blood was collected from the animals on days -5, -2 (pre-carboplatin), 1 (4 hr post carboplatin dosing), 3, 7, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 32 and 36. Blood samples (approximately 1.3 mL) were collected using a butterfly catheter from the cephalic vein. Blood was transferred to a tube treated with potassium EDTA, mixed by gentle inversion and used for measurement of blood parameters. Blood was centrifuged at 2000 x g, for about 15 minutes at 2 to 8°C within one hour of collection and divided into two approximately equal (0.25 mL) plasma aliquots. The plasma was stored at ≤ –70°C. The compounds, formulations, study design and hematology parameters that were used or measured are described in the tables below.

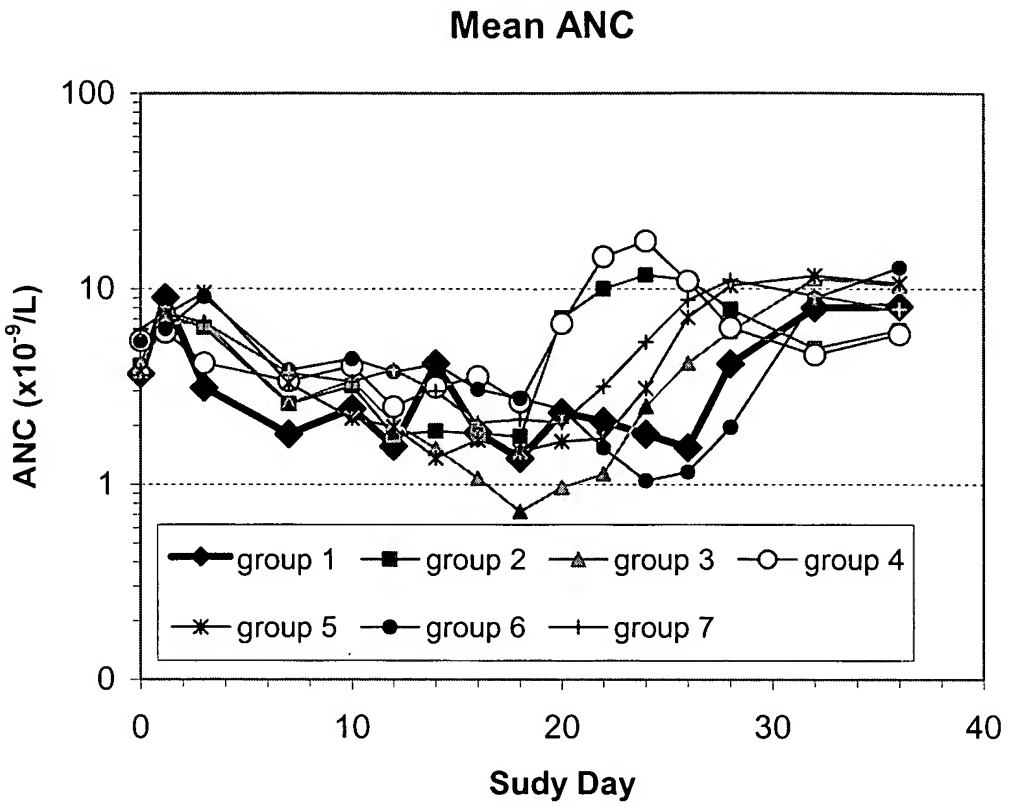
Group	Compound	Formulation	Excipients
1	none (vehicle control)	Solution	0.1% w/v carboxymethylcellulose, 0.9% w/v sodium chloride, 0.05% v/v phenol
2	3β,17β-dihydroxyandrost-5-ene	Micronized suspension	0.1% w/v carboxymethylcellulose, 0.9% w/v sodium chloride, 0.05% v/v phenol
3	3β,17β-dihydroxyandrost-5-ene	Micronized suspension	0.1% w/v carboxymethylcellulose, 0.9% w/v sodium chloride, 0.05% v/v phenol
4	3β,17β-dihydroxyandrost-5-ene	Nanosuspension	phosphate/glycerin buffer
5	3β-hydroxy-17β-aminoandrost-5-ene	Unmicronized suspension	0.1% w/v carboxymethylcellulose, 0.9% w/v sodium chloride, 0.05% v/v phenol
6	3β-hydroxy-17β-aminoandrost-5-ene	Unmicronized suspension	0.1% w/v carboxymethylcellulose, 0.9% w/v sodium chloride, 0.05% v/v phenol
7	3β,17β-dihydroxyandrost-5-ene	Nanosuspension	phosphate/glycerin buffer

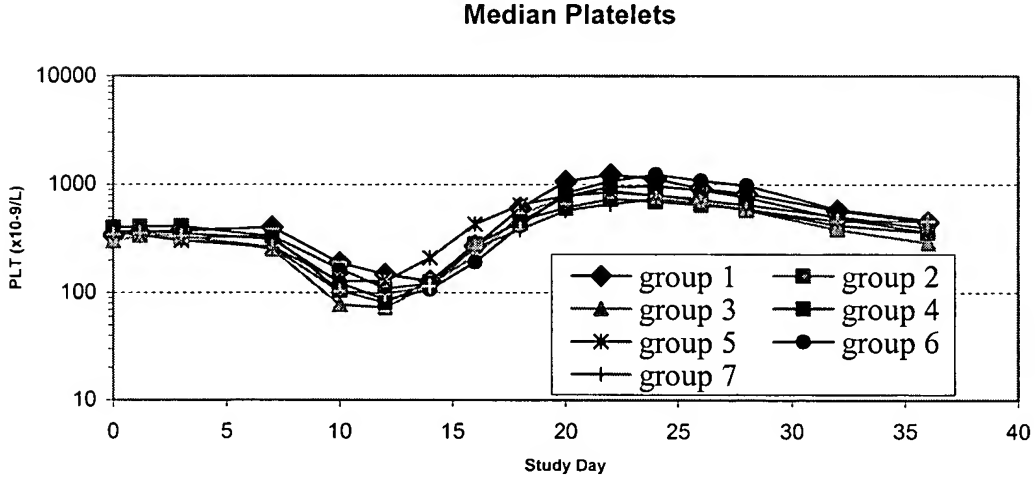
Group	Dose route	Dosing	Dosing days	Dose level (mg/kg)	Compound conc. (mg/mL)	Number of Animals	
						Male	Female
1	SC	Once on days 1, 2, 3, 4, 5	5	0	0	2	2
2	SC	Once on days 1, 2, 3, 4, 5	5	7.5	100	2	2
3	SC	Once on days 1, 3, 5, 7, 9	5	7.5	100	2	2
4	SC	Once on days 1, 8, 15	3	7.5	100	2	2
5	SC	Once on days 1, 2, 3, 4, 5	5	0.85	8.5	2	2
6	SC	Once on days 1, 8, 15	3	1.42	25	3	1
7	IV	Once on days 1, 8, 15	3	2.5	100	3	1

Hematology parameter	Method	Units
Erythrocyte count (RBC)	Light scattering	$x10^6/\mu L$
Leukocyte count (WBC)	Peroxidase/Basophil	$x10^3/\mu L$
Hematocrit value (HCT)	Calculation	%
Hemoglobin concentration (Hgb)	Cyanmethemoglobin	g/dL
Platelet count (PLT)	Light scattering	$x10^3/\mu L$
Mean platelet volume (MPV)	Light scattering/Calculation	fL
Mean corpuscular volume (MCV)	Light scattering/Calculation	fL
Mean corpuscular hemoglobin (MCH)	Light scattering/Calculation	Pg
Mean corpuscular hemoglobin concentration (MCHC)	Calculation	g/dL
Differential leukocyte count (Absolute and Percentage)	Peroxidase/Basophils	% and $x10^3/\mu L$

7. Results from the animals described in paragraph 6 showed faster neutrophil recovery in group 2, 4 and 5 animals compared to the group 1 controls. Platelet recovery in group 5 occurred sooner than recovery in group 1. Results that were obtained are shown in the table below. Enhanced neutrophil or platelet recovery was not observed in the group 8 animals because the dose was too low to elicit a response. Veterinary pathology analyses indicated that the cause of death in animals that died was primarily due to thrombocytopenia and bleeding accompanied by infection resulting from neutropenia. Some animals (< 15%) died from infection associated with thrombocytopenia and neutropenia.

Mean absolute neutrophil counts (ANC) and platelet (PLT) counts are shown in the graphs below.





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8. The compound 3β-hydroxy-17β-aminoandrost-5-ene was tested for its effect on chemotherapy-induced neutropenia and thrombocytopenia in cynomolgus monkeys. The blood cell deficiencies were induced by treatment of the animals with a single 40 mg/kg dose of carboplatin essentially as described above in paragraph 6. Three groups of animals were used. Group 1 was the vehicle control consisting of 4 animals (2 male, 2 female), group 2 consisted of four animals (2 male, 2 female) dosed with 2.5 mg/kg of unmicronized 3β-hydroxy-17β-aminoandrost-5-ene by subcutaneous injection on days 1, 2, 3, 4 and 5 and group 3 consisted of four animals (3 male, 1 female) dosed with 4.22 mg/kg of unmicronized 3β-hydroxy-17β-aminoandrost-5-ene by subcutaneous injection on days 1, 8 and 15.

Median absolute neutrophil counts (ANC) and platelet counts are shown in the tables and graphs below.

median ANC x 10^{-9}/L	day 0	1	3	7	10
group 1	5.4	13.1	3.3	1.4	4.9
group 2	6.2	11.4	13.1	6.7	7.3
group 3	8.6	7.7	15.4	3.0	12.2

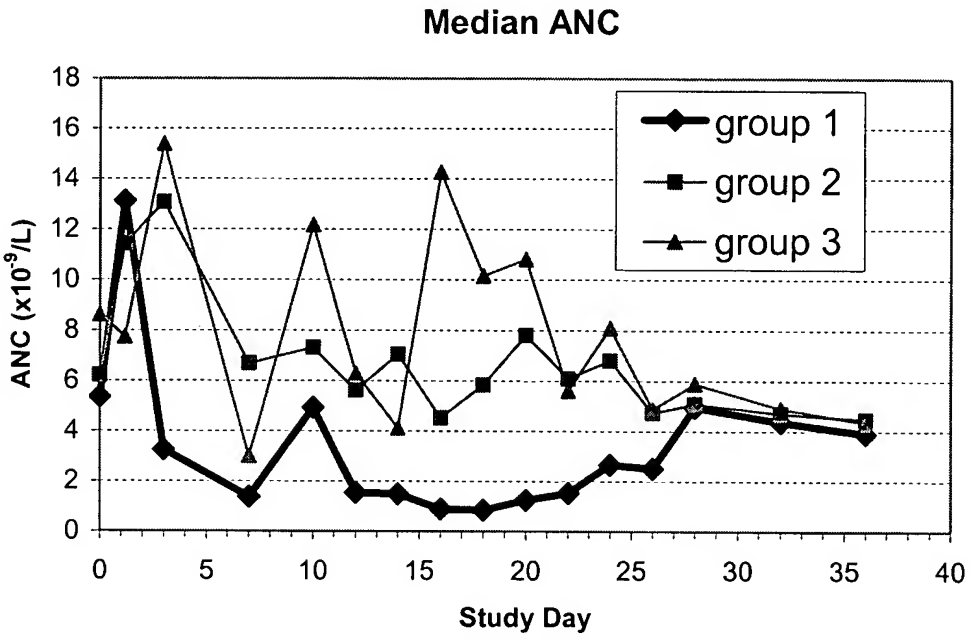
median ANC x 10^{-9}/L	day 12	14	16	18	20
group 1	1.5	1.5	0.9	0.9	1.3
group 2	5.6	7.1	4.5	5.8	7.8
group 3	6.3	4.1	14.3	10.2	10.8

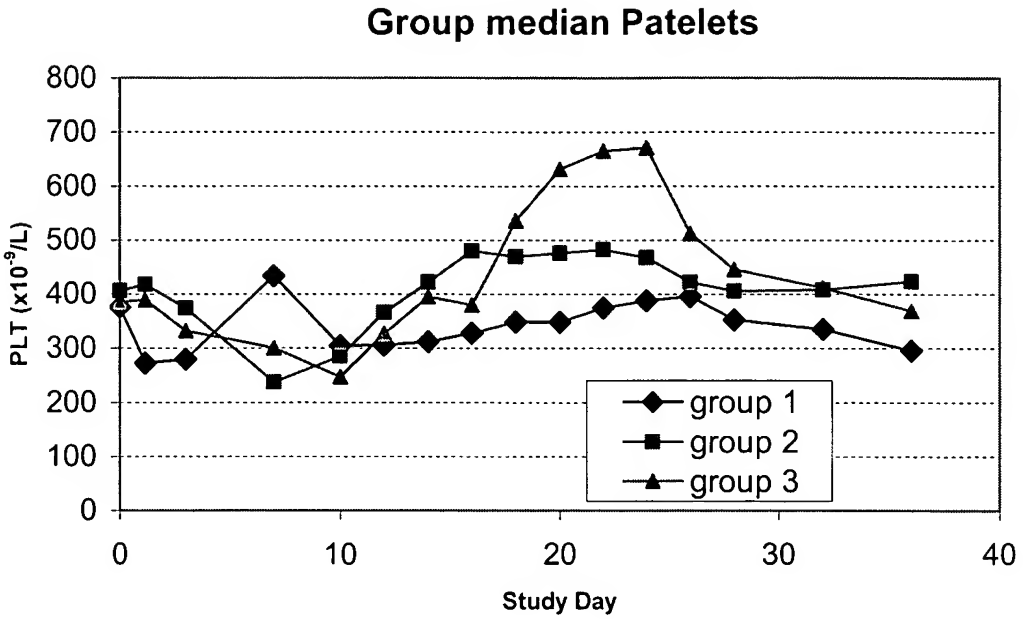
median ANC x 10^{-9}/L	day 22	24	26	28	32	36
group 1	1.5	2.7	2.5	5.0	4.4	3.9
group 2	6.1	6.8	4.7	5.1	4.7	4.5
group 3	5.6	8.1	4.9	5.9	4.9	4.3

median platelets x 10^{-9}/L	day 0	1	3	7	10	12
group 1	376	273	280	434	305	306
group 2	406.50	418	375	238	286	366
group 3	386	390	333	301	247	327

median platelets x 10^{-9}/L	day 14	16	18	20	22	24
group 1	312	328	349	348	375	388
group 2	422	480	470	476	483	468
group 3	395	380	536	632	666	672

median platelets x 10^{-9}/L	day 26	28	32	36
group 1	396	353	335	296
group 2	422	406	409	424
group 3	513	446	412	369





9. The effect of compounds on survival of lethally irradiated female B6D2F1 mice were compared to control animals treated with vehicle alone. In this model, animals were exposed to a lethal radiation dose and the failure of the animals to survive was due largely to thrombocytopenia and neutropenia and associated bleeding and infection. The animals were exposed to 10 Gy of total body γ-irradiation at 2.5 Gy/min using a ^{137}Cs source. Groups of 12 animals were used in a total of 5 groups. For Groups 1, 2, 3, and 5, test article was administered as a 100 μL volume, by subcutaneous injection, for three consecutive days, with the first dose administered 2 to 4 hours following exposure to radiation. For Group 4, test article was administered as a 50 μL volume, by intramuscular injection for three consecutive days. The formulation was a suspension containing 0.1% w/v carboxymethylcellulose, 0.9% w/v sodium chloride and 0.05% v/v phenol. The formulations were agitated to uniformly resuspend the compound before syringing, and injected into animals within a few minutes of drawing into the syringe to prevent settling.

10. The groups of animals described in paragraph 9 were treated as follows. Group 1 received vehicle only by daily subcutaneous injection for 3 consecutive days. Group 2 received 0.6 mg in 100 μL of a suspension of 3β,17β-dihydroxyandrost-5-ene by daily subcutaneous injection for 3 consecutive days. Group 3 received 3.0 mg in 100 μL of a suspension of 3β,17β-dihydroxyandrost-5-ene by daily subcutaneous injection for 3 consecutive days. Group 4 received 0.6 mg in 50 μL of a suspension of 3β,17β-dihydroxyandrost-5-ene by daily intramuscular injection for 3 consecutive days. Group 5 received 0.6 mg in 100 μL of a suspension of 3β-hydroxy-17β-aminoandrost-5-ene by daily subcutaneous injection for 3 consecutive days. Survival of the animals was monitored for 21 days after irradiation and the following results were obtained. The number of surviving animals is shown for day 6, 7, 12 and 21. The compounds increased the rate of survival of subjects that were exposed to an otherwise lethal dose of ionizing radiation. This data indicates that the compounds enhanced recovery of neutrophils and platelets.

	Day			
Group	6	7	12	21
1 vehicle control	12	11	4	1
2 0.6 mg SC	12	11	10	7
3 3.0 mg SC	12	12	9	7
4 0.6 mg IM	12	12	11	9
5 0.6 mg SC	12	12	12	11

11. Mice were exposed to a dose of ionizing radiation to induce a detectable cytopenia in the animals as measured by neutrophil counts. This analysis was used to characterize the effect of 3β,17β-dihydroxyandrost-5-ene, 3β,17α-dihydroxyandrost-5-ene, 3β,7β,17β-trihydroxyandrost-5-ene, 3,17-dioxoandrost-4-ene, 16α-fluoro-17-oxoandrost-5-en-17-one, 3β-hydroxy-16α-bromo-5α-androstan-17-one, 16α-fluoro-17α-hydroxyandrost-5-ene and 16α-fluoro-17β-hydroxyandrost-5-ene. Mice (female B6D2F1 or male C3H/HeN) received subcutaneous injections of the formula 1 compound in PEG-400 vehicle either 24 hours before or 1 hour after whole-body gamma-irradiation. Compound dosages ranged from 20 mg/kg to 320 mg/kg. The radiation dose was between 9

and 13 Gy for survival experiments. Survival of the animals was monitored. Animals treated with the compounds 16α-fluoro-17α-hydroxyandrost-5-ene, 16α-fluoro-17β-hydroxyandrost-5-ene, 3β,7β,17β-trihydroxyandrost-5-ene and 3β,17β-dihydroxyandrost-5-ene had an increased survival rate at 20 days compared to untreated control animals. The effects of the compounds on numbers of circulating neutrophils were examined using mice treated with 30 mg/kg of the compound 24 hours before a 3 Gy radiation dose. Blood was collected 2 days after irradiation, when neutrophils were significantly depressed. Animals treated with 3β,7β,17β-trihydroxyandrost-5-ene or 3β,17β-dihydroxyandrost-5-ene had increased levels of circulating neutrophils compared to vehicle-treated control mice.

12. Balb/c mice were treated with sufficient cyclophosphamide to induce neutropenia. This analysis was used to characterize the effect of compound treatment on the duration and severity of neutropenia. The animals were injected once with 200 mg/kg of cyclophosphamide and were then injected with compound once per day on days 1, 2, 3, 4 and 5 after cyclophosphamide was administered. Control animals received injections of vehicle without compound. Compounds the treated animals received were 5 mg/day of 3β,17β-dihydroxyandrost-5-ene, 3β,7β,17β-trihydroxyandrost-5-ene or 3β,17β-dihydroxy-7-oxoandrost-5-ene by subcutaneous injection. For animals that received the compounds, neutrophil counts at days 6 and 7 in the treated animals were higher than neutrophil counts in the control animals or, for 3β,17β-dihydroxy-7-oxoandrost-5-ene, the neutrophil count nadir was higher than in control animals. These results showed that the compounds ameliorated neutropenia by shortening the time to recovery or by reducing its severity.

13. As shown by the data described in the paragraphs above, the efficacy of the compounds varies with the formulation, dosage, dose regimen and route of administration that is used. In view of the data, I believe that compounds such as 3β-hydroxy-17β-aminoandrost-5-ene can be used without undue

experimentation to treat a range of blood cell deficiencies, including neutropenia, thrombocytopenia and anemia. Beneficial effects of treatment with the compounds include a reduction in the maximum severity (cell nadir) of cytopenia, a shorter time to recovery of cell counts to baseline or both.

14. I hereby declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such false statements may jeopardize the validity of the application or any patent issued thereon.

Date: Jan 17, 2006 By: Christopher L. Reading

Christopher L. Reading

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- Ph.D. in Biochemistry, U.C. Berkeley, 1977

- Postdoctoral Fellowship in Tumor Biology, U.C. Irvine, 1978-1980

- Faculty, M.D. Anderson Cancer Center, 1980-1993
  Tenured Associate Professor of Medicine, Department of Hematology
  Stem Cell Transplantation and Gene Therapy
  Four granted patents in bispecific monoclonal antibodies and devices

- SyStemix, Inc. 1993-1998
  Vice President for Product and Process Development 1996-1998
  Senior Management team involved in sale of SyStemix, Inc. to Novartis
  Senior Director of Cellular Purification 1993-1996
  IND for autologous stem cell isolation for cancer
  IND for in utero transplantation
  IND for stem cell gene therapy for HIV

- Novartis Biotechnology Development and Production 1997-1998
  Cell and Gene Therapy Strategy
  Immune Cell Therapy Strategy
  Technical Analyst for Mergers and Acquisitions in Cell and Gene Therapy
  Technical Analyst for Intellectual Property in Cell and Gene Therapy
  Technical Analyst for Business Development and Licensing
  REV123 HIV Gene Therapy International Project Team
  GTI/SyStemix Technical Research and Development Integration Team

- Hollis-Eden Pharmaceuticals 1998-Present
  Vice President for Scientific Development
  IND for 16α-bromoepiandrosterone treatment of HIV
  IND for 3β, 7β, 17β-androstenetriol in vaccination of the elderly
  International clinical trial development for HIV, Malaria, HBV, HCV
  Established collaborations in South Africa, Thailand, Singapore and Australia
  Frequent presentations to Investment Bankers and Wall Street

- International Scientific Reputation 1977-2000
  30 National and International Scientific Presentations
  77 publications in peer-reviewed journals
  18 invited journal articles
  20 invited book chapters
  National Science Foundation Advisory Committee for SBIR Grants
  Editorial Board, Journal Biological Response Modifiers
  Editorial Board, Molecular Biotherapy
  Peer-reviewed Grants and Contracts totaling over $2 Million
  Consultant to Government Agencies and Private Corporations

Published Articles in Peer-Reviewed Journals

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